T3 Wheat Tutorial – Primer Design

1. Predesigned primers for 2017_WheatCAP experiment

The PolyMarker program was used to design primers on all the markers in the 2017_WheatCAP experiment. This table below gives a good summary of the results. PolyMarker automates the design of genome specific primers. Primer3 is used to pick candidates then PolyMarker selects a primer pair with the highest specificity.

SNP_type	primer_type	total	percentage
		32466	2.92920590
homoeologous		3778	0.34086552
homoeologous	chromosome_nonspecific	616	0.05557786
homoeologous	chromosome_semispecific	10575	0.95411669
homoeologous	chromosome_specific	5202	0.46934421
non-homoeologous		189269	17.07656843
non-homoeologous	chromosome_nonspecific	170574	15.38983448
non-homoeologous	chromosome_semispecific	436538	39.38611726
non-homoeologous	chromosome_specific	259337	23.39836966

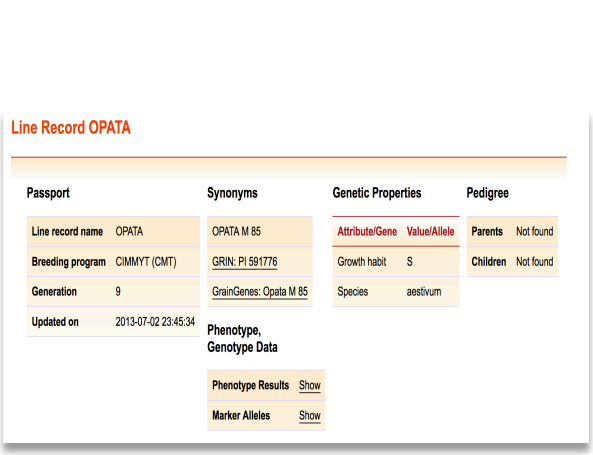
2. Three ways to access the designed primers in T3 Wheat

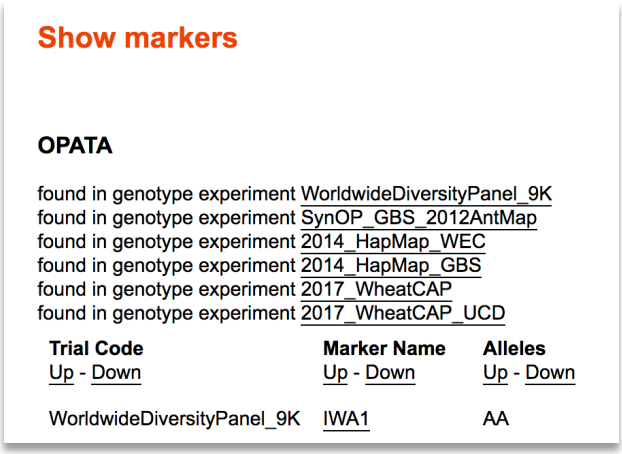
1. Go to Reports => JBrowse => RefSeq_v1. Make sure the “Primers 2017_WheatCAP” is selected in available tracks. Navigate to the region of interest then click on an item in the “Primers 2017_WheatCAP” track to view primer information. Within the popup box there is a “T3_link” to more information on the associated SNP.
2. Go to Select => Markers. Select “Wheat CAP 2017” Map, then chromosome, then enter a range, then “show markers”. Highlight up to 10 markers and click on the “Select markers” button. Then go to Reports => Designed Primers.
3. Go to Analyze => BLAST. Enter a marker sequence, select “RefSeq_v1”, then select “Basic Search”. On the results page click on the best hit in the “T3 JBrowse” column. In JBrowse click on an item in the “Primers 2017_WheatCAP” track.

Selecting markers not in WheatCAP_2017 experiment for primer design

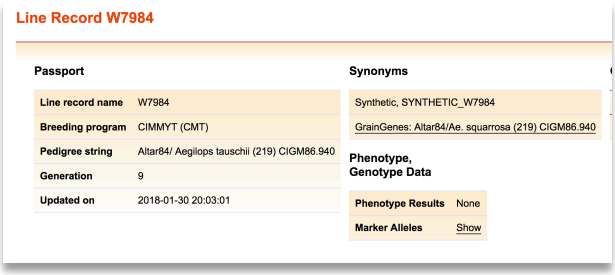
If you have two lines (germplasm) and you want to find which markers are polymorphic in those lines use the following procedure.

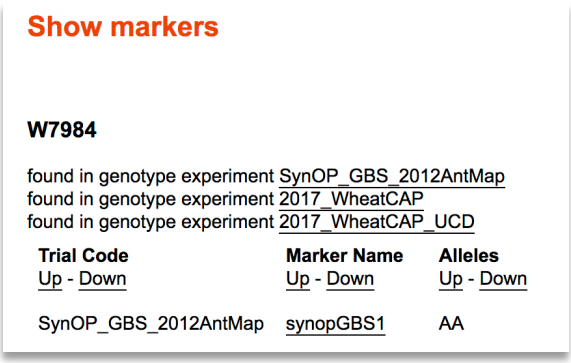
1. Select the first line (germplasm) using “Quick search”. Click on the Marker Alleles Show link. The web page will now show the genotype experiments where that line was genotype. Record these experiments.



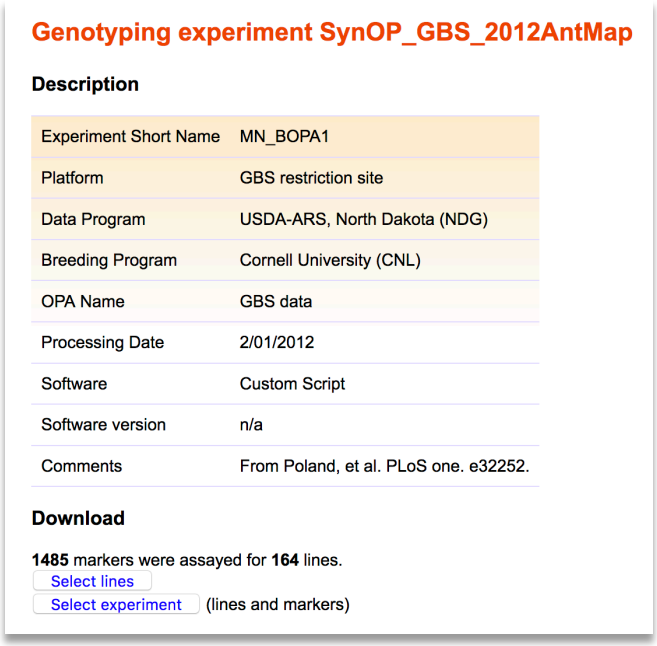


2. Select the second line using “Quick search”. Click on the Marker Alleles Show link. The web page will now show the genotype experiments where that line was genotype. Record these experiments.
3. Then find the common experiments that genotyped both lines.





4. Select one of those experiment either using “Quick search” or clicking on the link in “Show markers” page.
5. On the “Genotyping experiment” page click on the “Select experiment” button.



Genotyping experiment SynOP_GBS_2012AntMap

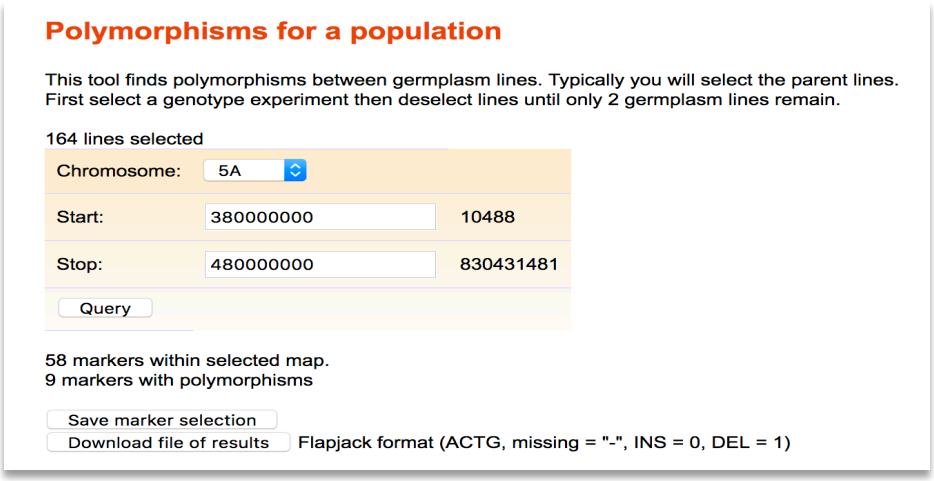
Description

Experiment Short Name	MN_BOPA1
Platform	GBS restriction site
Data Program	USDA-ARS, North Dakota (NDG)
Breeding Program	Cornell University (CNL)
OPA Name	GBS data
Processing Date	2/01/2012
Software	Custom Script
Software version	n/a
Comments	From Poland, et al. PLoS one. e32252.

Download

1485 markers were assayed for **164** lines.
Select lines
Select experiment (lines and markers)

4. Go to Select => Map and select the RefSeq v1.0 map
5. Go to Select => Subset Marker by Polymorphisms
6. Select Chromosome, Start, Stop, then Query
7. The output is the markers that are polymorphic. Click on “Save marker selection”.



Polymorphisms for a population

This tool finds polymorphisms between germplasm lines. Typically you will select the parent lines. First select a genotype experiment then deselect lines until only 2 germplasm lines remain.

164 lines selected

Chromosome:	5A	
Start:	380000000	10488
Stop:	480000000	830431481

Query

58 markers within selected map.
9 markers with polymorphisms

Save marker selection
Download file of results Flapjack format (ACTG, missing = "-", INS = 0, DEL = 1)

8. Click on the Markers link in the Quick Links section.
9. Download marker information
10. These results can be manually formatted then submitted to PolyMarker for design

Marker Information

name	type	A_allele	B_allele	synonym	mapped	lines genot
synopGBS70	SNP	A	T	synopGBS101348	Yes	326
synopGBS57	SNP	C	T	synopGBS101113	Yes	326
synopGBS380	SNP	A	G	synopGBS108413	Yes	326
synopGBS422	SNP	A	C	synopGBS109514	Yes	326
synopGBS440	SNP	A	G	synopGBS110285	Yes	326
synopGBS902	SNP	A	G	synopGBS121777	Yes	326
synopGBS1198	SNP	C	G	synopGBS125923	Yes	326
synopGBS147	SNP	C	T	synopGBS102216	Yes	326
synopGBS429	SNP	G	T	synopGBS109755	Yes	326

Download marker information